

probe B, said nucleic acid A binding probe B having a base sequence and a binding region which binds to nucleic acid A, and one or more nucleic acid A binding probes C, wherein said one or more nucleic acid A binding probes C, ~~in the aggregate~~, comprise a base sequence different from the base sequence of nucleic acid A binding probe B and an aggregate binding region wherein said aggregate binding region, which binds to nucleic acid A, is longer as compared with the binding region of probe B; and (b) determining the presence or amount of said triple stranded complex as an indication of the presence or amount of nucleic acid A.

32. The method according to claim 31 wherein said triple stranded complex contains only one nucleic acid A binding probe C.

33. The method according to claim 31 wherein said nucleic acid A binding probe B comprises a binding region of 4 to 10 bases.

34. The method according to claim 31 wherein said binding region of nucleic acid A binding probe B has an asymmetric base sequence.

35. The method according to claim 31 wherein said binding region of nucleic acid A binding probe B has a symmetric base sequence.

36. The method according to claim 31 wherein said aggregate binding region of the one or more nucleic acid A binding probes C has a length of at least 6 bases.

37. The method according to claim 31 wherein said triple stranded complex comprises two different nucleic acid A binding probes C which bind to different regions of nucleic acid A in the triple stranded region.

A/B

38. The method according to claim 37 wherein said two different nucleic acid A binding probes C of the triple stranded complex form an aggregate binding region which is comprised of two distinct triple helical binding regions, wherein each distinct triple helical binding region is formed from the binding of the two different probes C each to a distinct, non-overlapping, region on nucleic acid A.

39. The method according to claim 38 wherein said two different nucleic acid A binding probes C bind juxtaposed on nucleic acid A.

40. The method according to claim 38 wherein said triple stranded complex is at least six bases in length and each of the two different nucleic acid A binding probes C individually contribute at least one but less than eleven bases to said triple stranded complex.

A 41. The method according to claim 31 wherein said binding region of nucleic acid A binding probe B comprises only pyrimidine bases but the aggregate binding region of the one or more nucleic acid A binding probes C comprises at least one non-pyrimidine-base.

C 42. The method according to claim 31 wherein said nucleic acid A binding probe B is bound to nucleic acid A via Hoogsteen base pairing and the one or more nucleic acid A binding probes C are bound to nucleic acid A via Watson Crick base pairing.

1 43. The method according to claim 31 wherein at least one of said nucleic acid A binding probes is labeled and the presence of said label in the triple stranded complex is used for determining the presence or amount of the nucleic acid A.

V 44. The method according to claim 32 wherein at least one of said nucleic acid A binding probes has been chemically modified to destabilize triple helix formation occurring by either of: (a) two nucleic acid A binding probes B binding to one nucleic acid A; or (b) two of said nucleic acid A binding probes C binding with one nucleic acid A.

45. The method according to claim 31 wherein a first nucleic acid not to be determined is differentiated from said nucleic acid A by a difference in the base sequence located outside the binding region of the nucleic acid A binding probe B but within the aggregate binding region of the one or more nucleic acid A binding probes C.

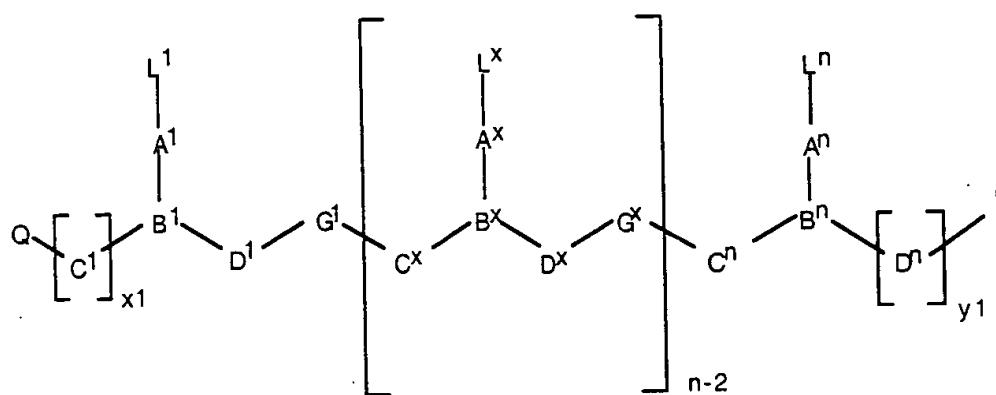
46. The method according to claim 31 wherein a first nucleic acid not to be determined is differentiated from said nucleic acid A by a difference in the base sequence located within the binding region of the nucleic acid A binding probe B.

47. The method according to claim 31 wherein a reaction mixture is used for forming the triple stranded complex, said reaction mixture containing a competitive probe D which can compete with at least one nucleic acid A binding probe C in binding to nucleic acid A, but which is incapable of participating in the formation of the triple stranded complex.

48. The method according to claim 31 wherein at least one of said nucleic acid A binding probes is a nucleic acid analogue.

49. The method according to claim 48 wherein said nucleic acid analogue is a peptide nucleic acid.

50. The method according to claim 31 wherein at least one of said nucleic acid A binding probes is a polymer of the general Formula I



Formula I

wherein

n is an integer of from at least 3,

x is an integer of from 2 to n-1,

each of L¹-Lⁿ is a ligand independently selected from the group consisting of hydrogen, hydroxy, C₁-C₄) alkanoyl, naturally occurring nucleobases, non-naturally occurring nucleobases, aromatic moieties, DNA intercalators, nucleobase-binding groups, heterocyclic moieties, reporter ligands and chelating moieties, wherein at least one of L¹-Lⁿ, preferably at least one of L²-Lⁿ⁻¹ is a non-nucleobase electron acceptor or a donor moiety and at least 2 of L¹-Lⁿ being a nucleobase binding group, or a naturally or non-naturally occurring nucleobase;

each of C¹-Cⁿ is (CR⁶R⁷)_y (preferably CR⁶R⁷, CHR⁶CHR⁷ or CR⁶R⁷CH₂) where R⁶ is hydrogen and R⁷ is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R⁶ and R⁷ are independently selected from the group consisting of hydrogen, (C₁-C₆) alkyl, aryl, aralkyl, heteroaryl, hydroxy, (C₁-C₆) alkoxy, (C₁-C₆) alkylthio, NR³R⁴ and SR⁵, where R³ and R⁴ are as defined below, and R⁵ is hydrogen, (C₁-C₆) alkyl, hydroxy, (C₁-C₆) alkoxy, or (C₁-C₆) alkylthio-substituted (C₁-C₆) alkyl or R⁶ and R⁷ taken together complete an alicyclic or heterocyclic system; or C¹-Cⁿ is CO, CS, CNR³;

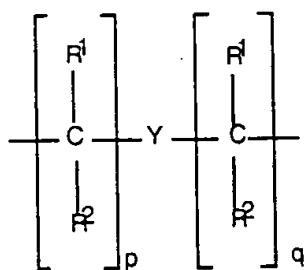
each of D^1 - D^n is $(CR^6R^7)_z$ (preferably CR^6R^7 , CHR^6CHR^7 or $CH_2CR^6R^7$) where R^6 and R^7 are as defined above;

each of y and z is zero or an integer from 1 to 10, the sum $y + z$ being at least 2, preferably greater than 2, but not more than 10;

each of G^1 - G^{n-1} is $-NR^3CO-$, $-NR^3CS-$, $-NR^3SO-$ or $-NR^3SO^2-$, in either orientation, where R^3 is as defined below;

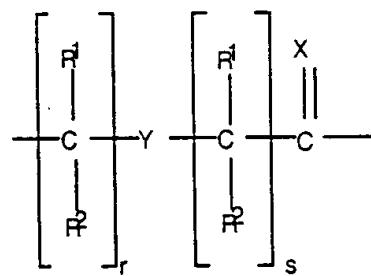
each of A^1 - A^n and B^1 - B^n are selected such that:

(a) A^1 - A^n is a group of formula (I/A), (I/B), (I/C) or (I/D), and B^1 - B^n is N or R^3N^+ ; or
(b) A^1 - A^n is a group of formula (I/D) and B^1 - B^n is CH;

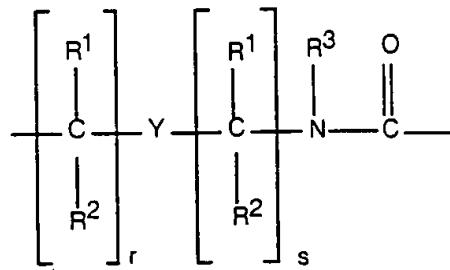


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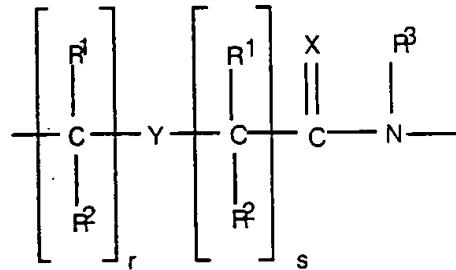
Formula I/A



Formula I/B



Formula I/C



Formula I/D

wherein:

X is O, S, Se, NR³, CH₂ or C(CH₃)₂;

Y is a single bond, O, S or NR⁴;

each of p and q is zero or an integer from 1 to 5, (the sum p+q being preferably not more than 5);

each of r and s is zero or an integer from 1 to 5, (the sum r+s being preferably not more than 5);

each R¹ and R² is independently selected from the group consisting of hydrogen, (C₁-C₄)alkyl which may be hydroxy- or (C₁-C₄)alkoxy- or (C₁-C₄)alkylthio-substituted, hydroxy, (C₁-C₄)alkoxy, (C₁-C₄)alkylthio, amino and halogen; and

each R³ and R⁴ is independently selected from the group consisting of hydrogen, (C₁-C₄)alkyl, hydroxy- or alkoxy- or alkylthio-substituted (C₁-C₄)alkyl, hydroxy, (C₁-C₆)-alkoxy, (C₁-C₆)-alkylthio and amino;

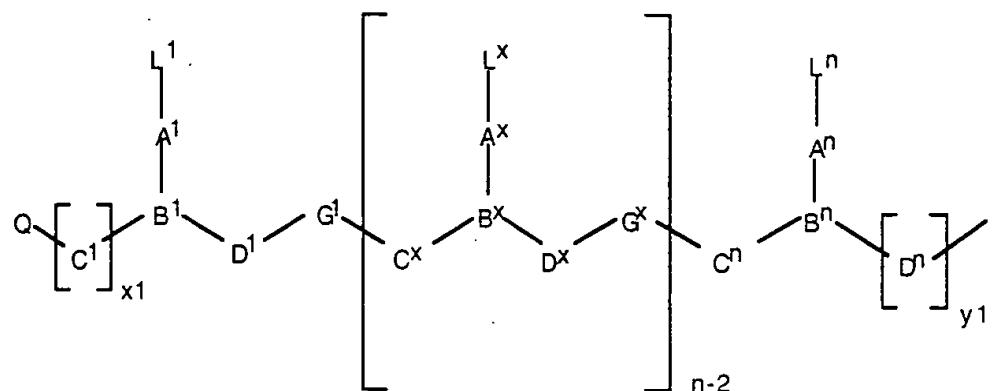
Q and I is independently selected from -CO₂H, -CONR'R'', -SO₃H or -SO₂NR'R'' or an activated derivative of -CO₂H or -SO₃H and -NR'R''

where R', R'' and R''' are independently selected from the group consisting of hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides, proteins, carbohydrates, lipids, steroids, nucleosides, nucleotides, nucleotide diphosphates, nucleotide triphosphates, oligonucleotides, including both

oligoribonucleotides and oligodeoxyribonucleotides, oligonucleosides and soluble and non-soluble polymers and as well as nucleic acid binding moieties and

each of x_1 and y_1 is an integer of from 0 to 10.

51. A method according to claim 31 wherein at least one of said nucleic acid A binding probes is a polymer of the general Formula I



Formula I

wherein

n is an integer of from at least 3,

x is an integer of from 2 to n-1,

each of L¹-Lⁿ is a ligand independently selected from the group consisting of hydrogen, hydroxy, C₁-C₄) alkanoyl; naturally occurring nucleobases; non-naturally occurring nucleobases, aromatic moieties, DNA intercalators, nucleobase-binding groups, heterocyclic moieties, reporter ligands and chelating moieties, wherein at least one of L¹-Lⁿ, preferably at least one of L²-Lⁿ⁻¹ is a non-nucleobase electron acceptor or a donor moiety and at least 2 of L¹-Lⁿ being a nucleobase binding group, or a naturally or non-naturally occurring nucleobase;

each of C¹-Cⁿ is (CR⁶R⁷)_y (preferably CR⁶R⁷, CHR⁶CHR⁷ or CR⁶R⁷CH₂) where R⁶ is hydrogen and R⁷ is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R⁶ and R⁷ are independently selected from the group consisting of hydrogen, (C₁-C₆) alkyl, aryl, aralkyl, heteroaryl, hydroxy, (C₁-C₆) alkoxy, (C₁-C₆) alkylthio, NR³R⁴ and SR⁵, where R³ and R⁴ are as defined below, and R⁵ is hydrogen, (C₁-C₆) alkyl, hydroxy, (C₁-C₆) alkoxy, or (C₁-C₆) alkylthio-substituted (C₁-C₆) alkyl or R⁶ and R⁷ taken together complete an alicyclic or heterocyclic system; or C¹-Cⁿ is CO, CS, CNR³;

each of D¹-Dⁿ is (CR⁶R⁷)_z (preferably CR⁶R⁷, CHR⁶CHR⁷ or CH₂CR⁶R⁷) where R⁶ and R⁷ are as defined above;

each of y and z is zero or an integer from 1 to 10, the sum y + z being at least 2, preferably greater than 2, but not more than 10;

each of G^1-G^{n-1} is $-NR^3CO-$, $-NR^3CS-$, $-NR^3SO-$ or $-NR^3SO^2-$, in either orientation, where R^3 is as defined below;

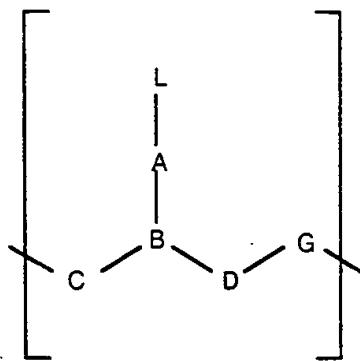
each of A^1-A^n and B^1-B^n are selected from: (la), (lb) or (lc) such that:

- (la): B^1-B^n is N and A^1-A^n is $-CO-(CH_2)_6-$
- (lb): B^1-B^n is N and A^1-A^n is $-CO-NR^3-(CH_2)_2-$
- (lc): B^1-B^n is CH and A^1-A^n is $-NR^3-CO-(CH_2)_2-$

Q and I is independently selected from $-CO_2H$, $-CONR'R''$, $-SO_3H$ or $-SO_2NR'R''$ or an activated derivative of $-CO_2H$ or $-SO_3H$ and $-NR'R''$

where R' , R'' and R''' are independently selected from the group consisting of hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides, proteins, carbohydrates, lipids, steroids, nucleosides, nucleotides, nucleotide diphosphates, nucleotide triphosphates, oligonucleotides, including both oligoribonucleotides and oligodeoxyribonucleotides, oligonucleosides and soluble and non-soluble polymers and as well as nucleic acid binding moieties and each of x_1 and y_1 is an integer of from 0 to 10.

52. The method according to claim 31 wherein at least one of said nucleic acid A binding probes comprise at least one monomer subunit of general Formula III



Formula III

each of L is a ligand independently selected from the group consisting of hydrogen, hydroxy, C₁-C₄)alkanoyl, naturally occurring nucleobases, non-naturally occurring nucleobases, aromatic moieties, DNA intercalators, nucleobase-binding groups, heterocyclic moieties, reporter ligands and chelating moieties, wherein at least one of L is a non-nucleobase electron acceptor or a donor moiety and at least 2 of L being a nucleobase binding group, or a naturally or non-naturally occurring nucleobase;

each of C is (CR⁶R⁷), where R⁶ is hydrogen and R⁷ is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R⁶ and R⁷ are independently selected from the group consisting of hydrogen, (C₁-C₆)alkyl, aryl, aralkyl, heteroaryl, hydroxy, (C₁-C₆)alkoxy, (C₁-C₆)alkylthio, NR³R⁴ and SR⁵,

where R^3 and R^4 are as defined below, and R^5 is hydrogen, (C_1-C_6) alkyl, hydroxy, (C_1-C_6) alkoxy, or (C_1-C_6) alkylthio-substituted (C_1-C_6) alkyl or R^6 and R^7 taken together complete an alicyclic or heterocyclic system; or C is CO, CS, CNR^3 ; each of D is $(CR^6R^7)_z$ where R^6 and R^7 are as defined above;

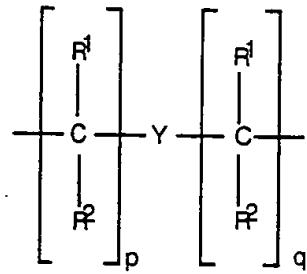
each of y and z is zero or an integer from 1 to 10, the sum $y + z$ being at least 2;

each of G is $-NR^3CO-$, $-NR^3CS-$, $-NR^3SO-$ or $-NR^3SO_2^-$, in either orientation, where R^3 is as defined below;

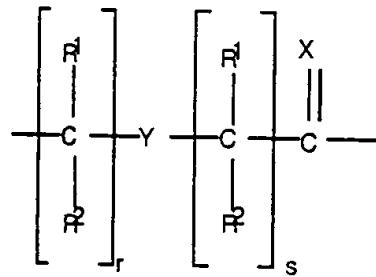
each of A and B are selected such that:

(a) A is a group of formula (I/A), (I/B), (I/C) or (I/D), and B is N or R^3N^+ ; or

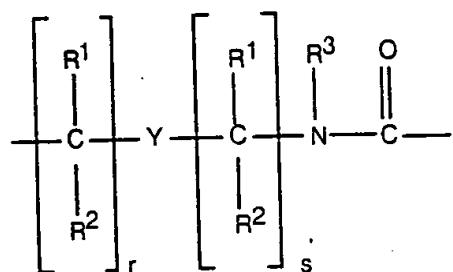
(b) A is a group of formula (I/D) and B is CH;



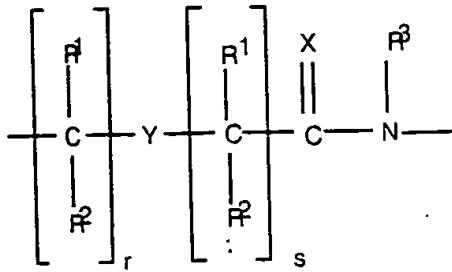
Formula I/A



Formula I/B



Formula I/C



Formula I/D

wherein:

X is O, S, Se, NR³, CH₂ or C(CH₃)₂;

Y is a single bond, O, S or NR^4 ;

each of p and q is zero or an integer from 1 to 5,

each of r and s is zero or an integer from 1 to 5,

each R¹ and R² is independently selected from the group consisting of hydrogen,

(C₁-C₄)alkyl which may be hydroxy- or (C₁-C₄)alkoxy- or (C₁-C₄)alkylthio-

substituted, hydroxy, (C_1-C_4) alkoxy, (C_1-C_4) alkylthio, amino and halogen; and

each R³ and R⁴ is independently selected from the group consisting of hydrogen,

(C₁-C₄)-alkyl, hydroxy- or alkoxy- or alkylthio-substituted (C₁-C₄)-alkyl, hydroxy, (C₁-C₆)-alkoxy, (C₁-C₆)-alkylthio and amino;

where R', R" and R'" are independently selected from the group consisting of hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides, proteins, carbohydrates, lipids, steroids, nucleosides, nucleotides,

nucleotide diphosphates, nucleotide triphosphates, oligonucleotides, including both oligoribonucleotides and oligodeoxyribonucleotides, oligonucleosides and soluble and non-soluble polymers and as well as nucleic acid binding moieties and each of x_1 and y_1 is an integer of from 0 to 10.

53. The method according to claim 31 wherein said triple stranded complex is more thermostable than a triple stranded complex formed by two nucleic acid A binding probes B binding with one nucleic acid A.

54. The method according to claim 32 wherein said triple stranded complex is more thermostable than a triple stranded complex formed from either of: (a) two nucleic acid A binding probes B binding with one nucleic acid A; or (b) two of said nucleic acid A binding probes C binding with one nucleic acid A.

55. A triple stranded complex comprising a nucleic acid A, a nucleic acid A binding probe B, said nucleic acid A binding probe B having a base sequence and a binding region which binds to nucleic acid A, and one or more nucleic acid A binding probes C wherein said one or more nucleic acid A binding probes C, in the aggregate, comprise a base sequence different from the sequence of nucleic acid A binding probe B and an aggregate binding region wherein said aggregate binding region, which binds to nucleic acid A, is longer as compared with the binding region of nucleic acid A binding probe B.

56. The complex according to claim 55 containing only one nucleic acid A binding probe C.

57. The complex according to claim 55 wherein said nucleic acid A binding probe B comprises a binding region of 4 to 10 bases.

58. The complex according to claim 55 wherein said aggregate binding region of the one or more nucleic acid A binding probes C has a length of at least 6 bases.

59. The complex according to claim 55 wherein said triple stranded complex comprises two different nucleic acid A binding probes C.

60. The complex according to claim 59 wherein said two different nucleic acid A binding probes C of the triple stranded complex form an aggregate binding region which is comprised of two distinct triple helical binding regions, wherein each distinct triple helical binding region is formed from the binding of the two different nucleic acid A binding probes C each to a distinct, non-overlapping, region on nucleic acid A.

61. The complex according to claim 60 wherein said two different nucleic acid A binding probes C bind juxtaposed on nucleic acid A.

62. The complex according to claim 55 wherein said binding region of nucleic acid A binding probe B comprises only pyrimidine bases but the aggregate binding region of the one or more nucleic acid A binding probes C comprises at least one non-pyrimidine base.

63. The complex according to claim 55 wherein said nucleic acid A binding probe B is bound to nucleic acid A via Hoogsteen base pairing and the one or more nucleic

acid A binding probes C are bound to nucleic acid A via Watson Crick base pairing.

64. The complex according to claim 55 wherein at least one of said nucleic acid A binding probes is labelled and the presence of said label in the triple stranded complex is used for determining the presence or amount of the nucleic acid A.

65. The complex according to claim 55 wherein at least one of said nucleic acid A binding probes is a nucleic acid analogue.

66. The complex according to claim 55 wherein said nucleic acid analogue is a peptide nucleic acid.

67. The complex according to claim 55 wherein said triple stranded complex is more thermostable than a triple stranded complex formed by two nucleic acid A binding probes B binding with one nucleic acid A.

68. The complex according to claim 56 wherein said triple stranded complex is more thermostable than a triple stranded complex formed from either of: (a) two nucleic acid A binding probes B binding with one nucleic acid A; or (b) two of said nucleic acid A binding probes C binding with one nucleic acid A.

69. A method of forming a triple stranded binding complex comprising reacting a nucleic acid molecule A with a nucleic acid A binding probe B, said nucleic acid A binding probe B having a base sequence and binding region which binds to nucleic acid A, and one or more nucleic acid A binding probes C, wherein said one or

more nucleic acid A binding probes C, in the aggregate, comprise a base sequence different from the sequence of nucleic acid A binding probe B and an aggregate binding region wherein said aggregate binding region, which binds to nucleic acid A, is longer as compared with the binding region of nucleic acid A binding probe B.

70. The method according to claim 69 wherein said triple stranded complex contains only one nucleic acid A binding probe C.

71. The method according to claim 69 wherein said nucleic acid A binding probe B comprises a binding region of 4 to 10 bases.

72. The method according to claim 69 wherein said aggregate binding region of the one or more nucleic acid A binding probes C has a length of at least 6 bases.

73. The method according to claim 69 wherein said triple stranded complex comprises two different nucleic acid A binding probes C.

74. The method according to claim 69 wherein said two different nucleic acid A binding probes C of the triple stranded complex form an aggregate binding region which is comprised of two distinct triple helical binding regions, wherein each distinct triple helical binding region is formed from the binding of the two different nucleic acid A binding probes C each to a distinct, non-overlapping, region on nucleic acid A.

75. The method according to claim 74 wherein said two different nucleic acid A binding probes C bind juxtaposed on nucleic acid A.

76. The method according to claim 69 wherein said binding region of nucleic acid A binding probe B comprises only pyrimidine bases but the aggregate binding region of the one or more nucleic acid A binding probes C comprises at least one non-pyrimidine base.

77. The method according to claim 69 wherein said nucleic acid A binding probe B is bound to nucleic acid A via Hoogsteen base pairing and the one or more nucleic acid A binding probes C are bound to nucleic acid A via Watson Crick base pairing.

78. The method according to claim 69 wherein at least one of said nucleic acid A binding probes is a nucleic acid analogue.

79. The method according to claim 69 wherein said nucleic acid analogue is a peptide nucleic acid.

80. The method according to claim 69 wherein said triple stranded complex is more thermostable than a triple stranded complex formed by two nucleic acid A binding probes B binding with one nucleic acid A.

81. The method according to claim 70 wherein said triple stranded complex is more thermostable than a triple stranded complex formed from either of: (a) two nucleic

acid A binding probes B binding with one nucleic acid A; or (b) two of said nucleic acid A binding probes C binding with one nucleic acid A.

82. A method for determining the presence or amount of a nucleic acid A, comprising:
(a) forming a triple stranded complex between said nucleic acid A, a nucleic acid A binding probe B, said nucleic acid A binding probe B having a base sequence and a binding region which binds to nucleic acid A, and two nucleic acid A binding probes C, wherein said two nucleic acid A binding probes C, in the aggregate, comprise a base sequence different from the base sequence of nucleic acid A binding probe B and an aggregate binding region wherein said aggregate binding region, which binds to nucleic acid A, is longer as compared with the binding region of nucleic acid A binding probe B; and (b) determining the presence or amount of said nucleic acid A by measuring for the presence or amount of said triple stranded complex.

83. The method according to claim 82 wherein said two nucleic acid A binding probes C of the triple stranded complex are different and form an aggregate binding region which is comprised of two distinct triple helical binding regions, wherein each distinct triple helical binding region is formed from the binding of the two different nucleic acid A binding probes C each to a distinct, non-overlapping, region on nucleic acid A.

84. The method according to claim 83 wherein said two different nucleic acid A binding probes C bind juxtaposed on nucleic acid A.